SURFACE MODIFICATION OF SELECTIVE LASER MELTED STRUCTURES USING SELF-ASSEMBLED MONOLAYERS FOR BIOMEDICAL APPLICATIONS

Jayasheelan Vaithilingam, Ruth D Goodridge*, Steven D Christie*[†]*, Steve Edmondson*[#] *and Richard J.M Hague**

*Department of Mechanical, Materials and Manufacturing Engineering, Nottingham University, UK.
[†]Department of Chemistry, Loughborough University, UK.
[#] Department of Materials, Loughborough University, UK.

Abstract

The potential of the selective laser melting (SLM) process to fabricate biomedical implants has been studied widely in recent years due to its ability to build complex and customised structures. Although SLM is capable of building almost any desired geometry, the surface properties of the produced parts are not controlled by the process and may not be suitable for many applications. Hence in this study, the coating of self-assembled monolayers (SAMs) onto SLM fabricated Ti6Al4V structures to modify surface chemistry has been studied. 16-phosphanohexadecanoic acid monolayers were used to modify SLM as-fabricated (SLM-AF) and mechanically polished (SLM-MP) surfaces. X-ray photoelectron spectroscopy (XPS) and static water contact angle measurements confirmed the formation of monolayers on these surfaces. The covalently bonded monolayers were found to be stable for up to six weeks immersion in Tris-HCl buffer solution. Stability of monolayers on SLM-AF and SLM-MP was not significantly different. This study demonstrates the possibility of forming stable phosphonic acid monolayers on SLM fabricated Ti6Al4V surfaces.

Keywords: Selective laser melting (SLM); self-assembled monolayers (SAMs);

1. Introduction

Although biomedical implants have improved the quality of life for countless people, they still impose unique challenges on current manufacturing processes[1]. The ability to achieve customised and complex geometries of biomedical implants with the current manufacturing techniques including moulding, die casting and other subtractive manufacturing process is limited. Post-implant complications that arise due to the failure of implants within the biological environment have been experienced since the introduction of these devices[2].

Although many different biomaterials (polymers, metals, ceramics and composites), fabrication techniques (such as moulding, die casting and laser cutting) and surface modification techniques (biocompatible material coating, surface polishing, drug loading etc.) have been employed to improve the biocompatibility of the implant, there are still constraints in achieving this. Even with the best biomaterial possessing adequate material properties, there are possibilities for the failure of an implant due to faulty mechanical design or inappropriate application of the implant. Also inadequate mechanical properties (e.g. elastic modulus, yield strength, tensile strength) can doom an implant to failure[2–5].

Although mechanical properties of implants are very important for avoiding post-implant complications, surface properties including surface texture, surface energy, surface charge and the stability of surface oxide layer have also gained considerable attention[6,7]. Conventional methods used to fabricate metal implants (e.g. laser cutting, die casting) have limitations for customisation, accessible geometries and control of the surface of the fabricated part. Although there are numerous surface modification techniques (including coating with biocompatible materials or drug-eluting polymer) available to reduce post-implant complications, most of them do not have precise control over the surface chemistry[8–10].

Selective laser melting (SLM), a metal-based additive manufacturing (AM) technique, is of particular interest due to its capability to fabricate functional components having mechanical properties comparable to those of the bulk materials[11]. The ability of SLM to build complex parts from 3D designs (including well defined porous structures) offers the possibility of customisation of biomedical implants. The potential for using SLM to fabricate biomedical implants has been discussed in more detail in the literature[12,13].

Self-assembled monolayers (SAMs) are composed of molecules with a head group which can attach to the surface to be modified and a tail group containing the functionality which is desired to be displayed on the surface. The ability of SAMs to precisely control surface chemistry with relatively simple and inexpensive processing, has led to numerous suggested applications[14], including biomedical applications[10,15–19].

In this paper, the combination of SLM with surface modification using monolayers has been evaluated, with the hope that in future, combining customised and complex geometries with surface chemistry control could reduce post-implant complications. A grade 5 titanium alloy (Ti6Al4V) was processed in SLM and surface modified using phosphonic acid monolayers. Since some biomedical applications (such as require rough surfaces while others require a smooth surface, surface modification of SLM fabricated Ti6Al4V structures possesing both rough and smooth surfaces has been evaluated[6,20]. The stability of phosphonic acid SAMs on these SLM fabricated surfaces has also been examined.

2. Materials and Methods

2.1. Materials

Ti6Al4V - a grade 5 titanium alloy gas-atomised powder with an average particle size distribution (volume weighted) of 33.35µm was supplied by LPW Technology Ltd., Lymm, UK. 16-phosphanohexadecanoic acid (16-PhDA) SAMs used for the surface modification was purchased from Sigma-Aldrich, Dorset, UK. Tetrahydrofuran (THF), hydrogen peroxide (30%) and sulphuric acid (98%) used for the cleaning and surface modification steps were purchased from Sigma-Aldrich, UK. Silicon carbide grits, polishing cloth and the extender solutions were supplied by Buehler (Buehler-met® II).

2.2. Methods

2.2.1. Design and fabrication

CAD Model

A plate with dimensions of 10x10x3mm was designed using Magics 14.1 (Materialise) software. This design was then replicated to fabricate the required number of samples simultaneously in the SLM machine using Ti6Al4V powders.

Selective Laser Melting

SLM of Ti6Al4V to fabricate the plates was conducted using an AM 250 laser melting machine (Renishaw, UK). A detailed description of the SLM process can be found in literature[21]. Ti6Al4V plates were fabricated using a constant laser power of 200W and a scan speed and layer thickness of 200mm/s and 50 µm respectively.

2.2.2. Surface preparation

Among the 24 plates built using SLM, 12 samples were used as-fabricated and the other 12 samples were mechanically polished using a series of silicon carbide grits (220µm, 400µm, 600µm, 800µm and 1200µm diameter, using 5-7 mins polishing with each). These surfaces were then polished using a polishing cloth with 6µm and 1µm diamond paste (3 mins each). Among the 12 samples in both the batches, two were kept as unmodified controls and the rest were surface modified using 16-phosphanohexadecanoic acid monolayers. Throughout this study, the as fabricated SLM plates will be referred as SLM-AF and mechanically polished SLM plates will be referred as SLM-MP.

Cleaning

The SLM as-fabricated (SLM-AF) and mechanically polished (SLM-MP) plates were cleaned by immersing in a mixture of sulphuric acid, hydrogen peroxide and water in the ratio of 1:1:5 respectively for 15 mins. These samples were then ultrasonicated in deionised water for 30 mins, fresh deionised water for a further 30 mins and dried. The samples were finally rinsed in THF and immediately immersed into the SAM solution.

2.2.3. Self-assembled monolayer formation

SAMs were coated on the SLM-AF and SLM-MP Ti6Al4V surfaces using solution immersion deposition following a previously reported procedure[17]. A 1 mM solution of 16-phosphonohexadecanoic acid $[(HO)OCH_2(CH_2)_{13}PO(OH)_2]$ in THF was prepared. After 24 h, the samples were removed from the THF and any residual solution was allowed to evaporate in air. Without rinsing, the samples were immediately transferred to an oven maintained at 120°C. After 24 h, the SAM-coated specimens were removed from the oven and allowed to cool to room temperature before ultrasonicating in THF and deionised water for 1 min each.

2.2.4. Stability Studies

16-PhDA coated SLM-AF and SLM-MP specimens were immersed in 20ml of 10 mM Tris-HCl buffer solution (TBS) at pH 7.4 and incubated at 36.5°C for up to 42 days (6 weeks). The samples were removed from the TBS solution at 7, 14, 28 and 42 days then ultra-sonicated with deionised water for 1 min and dried before characterisation.

2.2.5. Surface Characterisation

Scanning electron microscopy (SEM)

A LEO 440 SEM was used to obtain images of the surface morphology of SLM fabricated Ti6Al4V plates. The SEM was operated at an extra-high tension (EHT) power supply of 10kV. Images of the surfaces were obtained at the magnifications including 150x, 250x and 500x.

Surface profilometry

An Alicona InfiniteFocus® optical 3D measurement device G4f (serial number 017010809808) was used to measure the surface roughness (Ra) of the SLM-AF and SLM-

MP fabricated Ti6Al4V plates. 174.76µm square area was used to obtain the surface roughness pattern. The in-built software within the Alicona InfiniteFocus® surface profilometer was used to process the acquired data from the equipment.

Contact angle measurement

Static water contact angles of both SLM-AF and SLM-MP were measured before and after surface modification using SAMs. A drop volume of 2µl was placed on the surface and images were acquired using a camera (allowing the drop to settle for approximately 5 sec) in three distinct spots within the sample surface and averaged. The contact angles formed by the water drop on the surface were measured using ImageJ software.

X-ray photoelectron spectroscopy (XPS)

Surface chemistry of the SLM fabricated samples was probed using XPS (VG ESCALAB Mk l). Using aluminium (Al) Kα radiation at 8kV, high resolution spectra of all detected elements were collected at a pass energy of 100 eV. The relative intensities of the detected element peaks in low resolution spectra was used for atomic composition and high resolution spectra was used for looking at fine peak structure.

3. Results and Discussion

Figure 1 shows an SEM micrograph of an SLM-AF surface. It can be observed that the Ti6AL4V surface is rough due to the sintering of particles from the surrounding powder bed to the surface of the part. Figure 2 shows 3D profile of the SLM-AF surface obtained using a surface profilometer. The observed surface roughness (Ra) was 17.6 ± 3.7 µm. The presence of partially melted particles on the part surface is inevitable in SLM, although the magnitude of this problem is dependent on laser power, SLM process parameters and the materials properties of the powder [11].

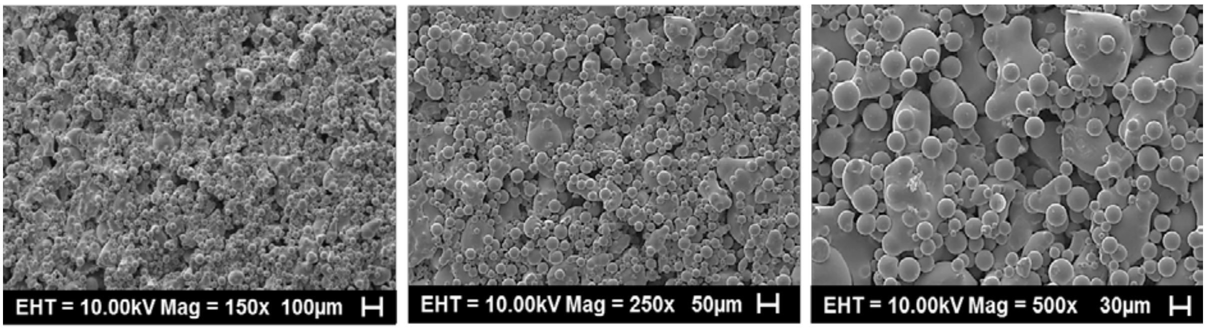

Figure 1 SEM images at various magnifications of the surface morphology of an as-fabricated Ti6Al4V surface on a plate made by SLM.

Previous studies on the attachment of monolayers were performed mostly on polished surfaces and a very few were performed on the rough surface[9,10,15–17,22,23]. For biomedical use, some applications require a smooth surface (such as stents) and some require a porous surface to promote cell adhesion and tissue integration/regeneration[6]. During our literature review, we have been unable to find any prior examples of the attachment of monolayers to an SLM fabricated surface. This study demonstrates the first example of the attachment of SAMs to a rough SLM fabricated Ti6Al4V surface and compares the stability of monolayers obtained for both SLM-AF (rough surface) and SLM-MP (mechanically polished) surface. The surface roughness of the SLM-MP after polishing was measured to be 0.586 ± 0.003 µm.

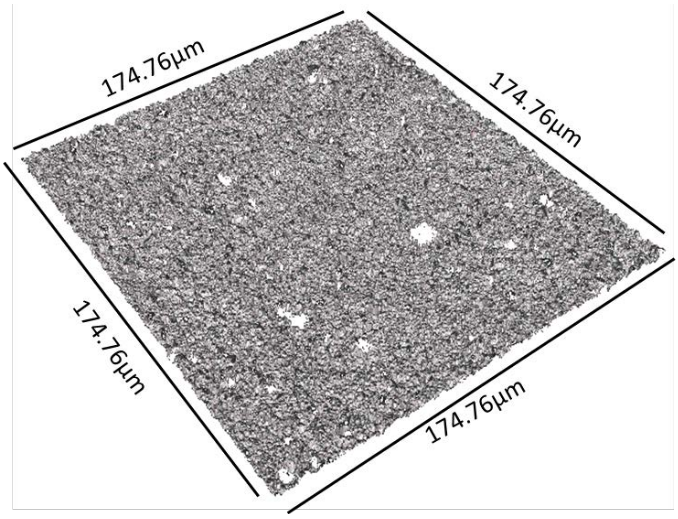

Figure 2 Surface Topography of SLM as-fabricated Ti6Al4V surface obtained using Alicona. The measured surface area was 174.76µm.

Since one batch of the samples was rough (SLM-AF), the commonly used techniques to characterise SAMs including atomic force microscopy (AFM) and ellipsometry could not be employed in this study. XPS and contact angle measurements were used to confirm the attachment and stability of the monolayers. Because SAMs with carboxylic group was found desirable for biomedical applications including osseointegration, 16-PhDA was used in this study to modify the SLM-AF and SLM-MP Ti6Al4V surfaces[24]. Figure 3 shows the XPS spectra of the SLM-AF and SLM-MP Ti6Al4V surfaces before and after 16-PhDA SAM coating. A phosphorous (P2p) peak can be observed at 133.2 eV, in agreement with the previous studies showing the presence of a metal phosphonate (P2p) peak at 133.2 eV after the attachment of phosphonic acid monolayers to metal surfaces[16–18,22,25].

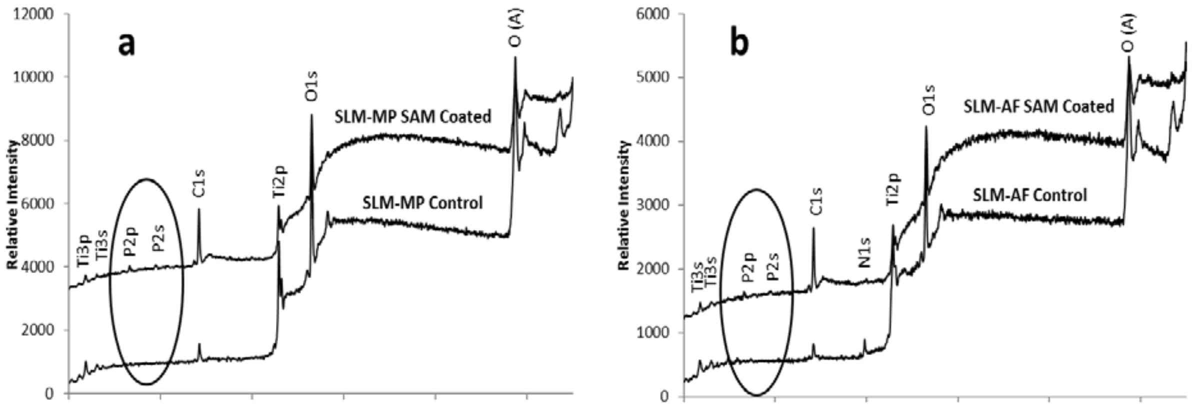

Figure 3 XPS spectra before and after surface modification of a) SLM-AF and b) SLM-MP by adsorption of a 16-PhDA monolayer.

The atomic composition of the Ti6Al4V surface before and after surface modification is given in Table 1. It can be observed that there is a significant increase in the carbon content after surface modification for both SLM-AF and SLM-MP. This increase in the carbon is consistent with the presence of the 16 carbon atoms of 16-PhDA. Also after surface modification, a significant amount of phosphorous was observed on the SAM-modified surface for both SLM-AF and SLM-MP. The atomic percentage of phosphorous is much less than that of carbon, consistent with the presence of only one phosphorous atom in 16-PhDA.

Table 1 Percentage composition of elements before and after surface modification

	Percentage Composition			
Elements	*SLM-AF before SAM attachment*	*SLM-AF after SAM attachment*	*SLM-MF before SAM attachment*	*SLM-MP after SAM attachment*
C	18.3	59.5	22	55.7
N	8.7	0	0	0
O	47.8	28.7	55.7	32.7
Ti	24.5	7.2	19	7.1
Al	0.7	0	3.3	0
P	0	4.6	0	4.5

The C:P atomic ratio is approximately 13:1 for SLM-AF and approximately 12:1 for SLM-MP. A ratio of 16:1 would be expected, and differences are likely due to errors in the integration of the small phosphorous peak. The observed aluminium (SLM-MP) and nitrogen (for SLM-AF) on the Ti6Al4V surfaces before SAM attachment disappeared after SAM formation. The observed nitrogen might be due to contamination of the surface during surface characterisation.

Also the concentration of titanium and oxygen observed before SAM attached decreased after surface modification, since the limited penetration depth of XPS is now sampling less of the underlying substrate. The detection of titanium and oxygen through the 16-PhDA layer confirms that this layer is thin (i.e. less than the typical XPS sampling depth of 5-10 nm). All observed changes in the surface chemistry of the SLM-AF and SLM-MP surfaces are consistent with the formation of SAMs on the SLM fabricated Ti6AL4V surfaces.

The contact angle formed by a liquid at an interface can provide a measure of surface wettability, SAM order and uniformity[16]. Figure 4 shows the surface wettability of SLM-AF and SLM-MP surfaces after different surface treatments. It can be observed that the contact angle reduced after surface cleaning. The high contact angle value before surface cleaning is likely due to the presence of contaminants on the surface. The important and problematic contaminants are hydrocarbons (oils from pumps, skin etc.) impurities on the material surface and some polymeric contaminants. These contaminants are mainly due to manual handling and exposure to the atmosphere[3,26].

On cleaning, these contaminants were effectively removed from the surface thus yielding lower contact angles for both SLM-AF and SLM-MP surfaces. After surface modification using SAMs, the contact angle reduced further. This was because the employed SAMs were hydrophilic, displaying a carboxylic acid (COOH) group. This further supports the formation of monolayer on both the SLM-AF and SLM-MP surfaces. Differences between the measured contact angles for the two surfaces are most likely due to the effect of the very different surface roughnesses.

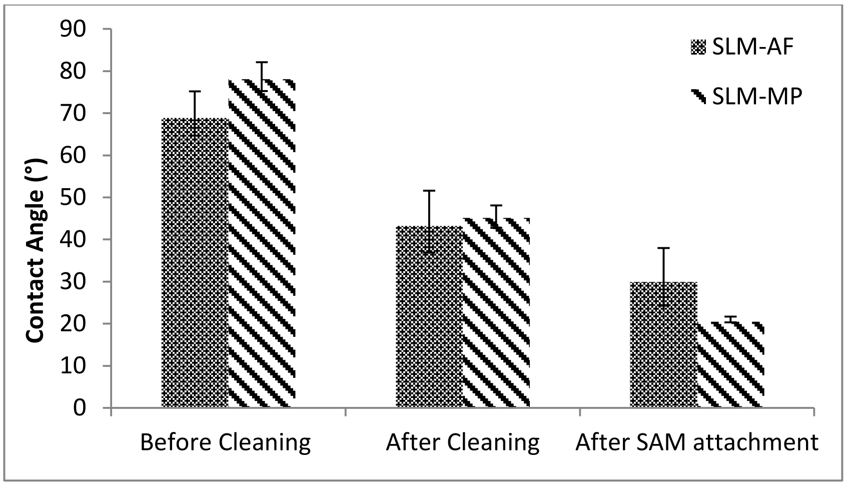

Figure 4 Static water contact angle measurements on SLM-AF and SLM-MP surfaces, after cleaning and SAM attachment.

Stability Studies

The stability of the phosphonic acid SAMs on SLM-AF and SLM-MP surfaces was examined by immersing the SAM-coated samples in TBS. Figure 5 shows the high resolution XPS spectra obtained for SLM-AF and SLM-MP after different soaking times in TBS. From the spectra it can be observed that the metal phosphonate peak at 133.2 eV stays at the same binding energy for both SLM-AF and SLM-MP surfaces after immersion in TBS. The covalently bonded phosphonic acid SAMs were observed to be stable for 28 days on both SLM-AF and SLM-MP surfaces[27]. Desorption of the monolayers were found to occur after 4 weeks; however, a small amount of phosphorous was observed at the end of six weeks.

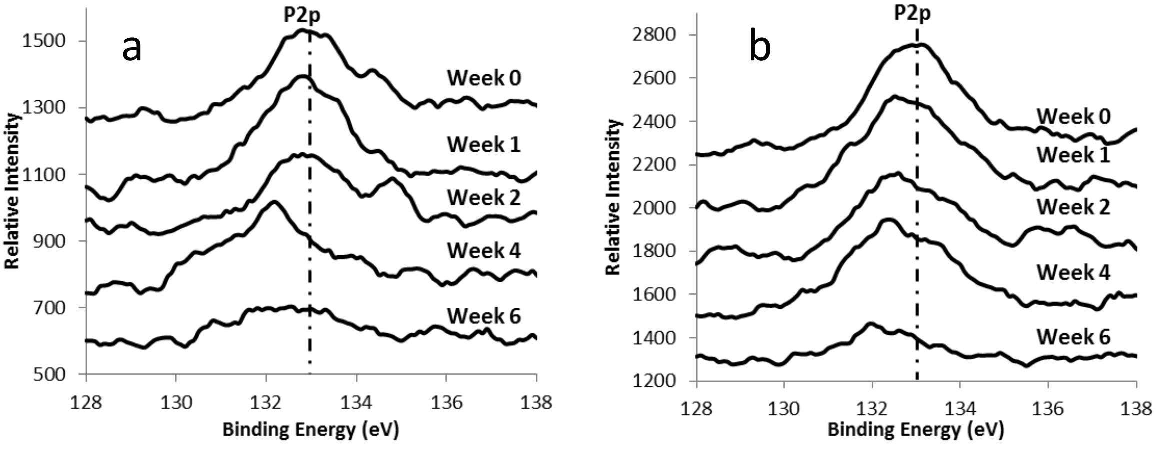

Figure 5 XPS spectra for the in-vitro stability of SAMs on a) SLM-AF and b) SLM-MP surfaces after soaking in TBS for various times.

Static contact angles were measured to examine the wettability of the SAM coated samples after immersion into TBS and are shown in Figure 6. It can be noted from the figure that the contact angles measured for the SLM-AF samples had a high standard deviation compared to the SLM-MP samples. This may be due to the surface roughness of the SLM-AF samples.

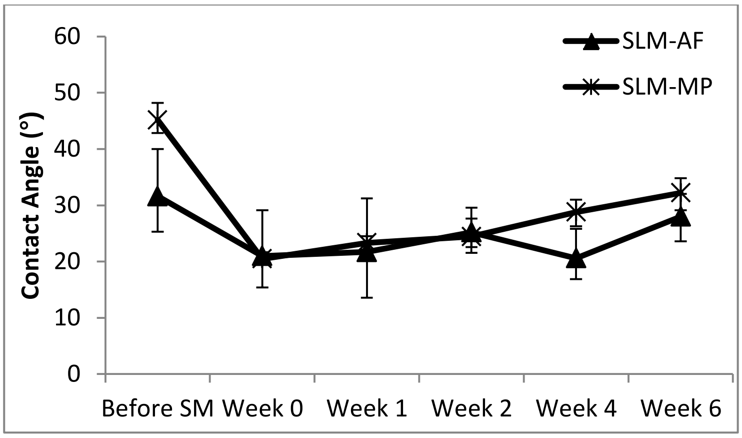

Figure 6 Static water contact angle measurements on SLM-fabricated surfaces are immersion for different times in TBS solution. “Before SM” refers to samples before surface modification using SAMs and “Week 0” measurements made immediately after surface modification.

The slight increase with time for the SLM-MP sample is consistent with monolayer desorption, although the effect is small. The effect of roughness also makes these results difficult to interpret; however, the surfaces of both SLM-AF and SLM-MP remained highly wettable.

4 Conclusion

SLM fabricated Ti6Al4V samples were observed to be rough and porous due to partially melted particles on the surface. The as-fabricated SLM surface (SLM-AF) and mechanically polished SLM surface (SLM-MP) were modified using 16-PhDA monolayers. XPS and contact angle measurements were consistent with the formation of monolayers. The surface roughness of the SLM-AF samples did not affect the monolayer formation significantly. The stability of these attached monolayers was examined in TBS for at time intervals of 1, 2, 4 and 6 weeks. 16PhDA monolayers were found to be stable under these conditions on both SLM-AF and SLM-MP surface for more than four weeks before it desorbed from the surface. However, a small amount of phosphorous was still observed on both SLM-AF and SLM-MP surface after 6 weeks.

The rough nature of the SLM-AF surface did not have a significant effect on the stability of the attached. Thus the possibility of attaching monolayers to selective laser melted Ti6Al4V samples and the stability of phosphonic acid monolayers on SLM-AF and SLM-MP surfaces has been demonstrated. Integration of SLM with surface modification using SAMs can potentially address some current challenges in biomedical applications. Also to extent further from biomedical applications, automotive/aeronautical parts made by SLM could also be surface-modified using SAMs to possess anti-microbical activity. Future work will involve drug/protein functionalisation of the monolayers followed by in-vitro studies with cells and stimulated body fluids to study the biocompatibility of surface modified parts.

References

1. Grillo HC. *Surgery of the trachea and bronchi*. Pmph USA Ltd; 2004.

2. Freitag L. Airway stents. *European Respiratory Monograph*. 2010:190-217.

3. Ratner BD. *Biomaterials science: an introduction to materials in medicine*. second edi. (Ratner BD, Hoffman A s, Schoen FJ, Lemons JE, eds.). London: Elsevier Academic Press; 2004.

4. Wood D. Tracheal and Bronchial Stenting. In: Grillo H, ed. *Surgery of the Trachea and Bronchi*. Ontario: BC Decker Inc, Hamilton, Ontario; 2004:763-790.

5. Stoeckel D, Pelton A, Duerig T. Self-expanding nitinol stents: material and design considerations. *European radiology*. 2004;14(2):292-301.

6. Paital SR, Dahotre NB. Calcium phosphate coatings for bio-implant applications: Materials, performance factors, and methodologies. *Materials Science and Engineering: R: Reports*. 2009;66(1-3):1-70.

7. Castner DG, Ratner BD. Biomedical surface science: Foundations to frontiers. *Surface Science*. 2002;500(1-3):28-60.

8. Mani G, Feldman MD, Patel D, Agrawal CM. Coronary stents: A materials perspective. *Biomaterials*. 2007;28(9):1689-1710.

9. Mani G, Johnson DM, Marton D, et al. Stability of self-assembled monolayers on titanium and gold. *Langmuir : the ACS journal of surfaces and colloids*. 2008;24(13):6774-84.

10. Torres N, Oh S, Appleford M, et al. Stability of antibacterial self-assembled monolayers on hydroxyapatite. *Acta biomaterialia*. 2010;6(8):3242-55.

11. Kruth J, Badrossamay M, Yasa E, et al. Part and material properties in selective laser melting of metals. In: *16th International Symposium on Electromachining*.; 2010:1-12.

12. Vandenbroucke B, Kruth J-P. Selective laser melting of biocompatible metals for rapid manufacturing of medical parts. *Rapid Prototyping Journal*. 2007;13(4):196-203.

13. Kruth J, Vandenbroucke B, Vaerenbergh JV, Naert I. Rapid Manufacturing of Dental Prostheses by means of Selective Laser Sintering / Melting. *Implant Dentistry*. 2005;(2).

14. Schwartz DK. Mechanisms And Kinetics of Self -Assembled Monolayer Formation. *Annual Review of Physical Chemistry*. 2001;52:107-37.

15. Mahapatro A, Johnson DM, Patel DN, et al. The use of alkanethiol self-assembled monolayers on 316L stainless steel for coronary artery stent nanomedicine applications: an oxidative and in vitro stability study. *Nanomedicine: nanotechnology, biology, and medicine*. 2006;2(3):182-90.

16. Bhure R, Abdel-Fattah TM, Bonner C, Hall F, Mahapatro A. Stability of Phosphonic Self Assembled Monolayers (SAMs) on Cobalt Chromium (Co-Cr) Alloy under Oxidative conditions. *Applied surface science*. 2011;257(13):5605-5612.

17. Kaufmann C, Mani G, Marton D, Johnson D, Agrawal CM. Long-term stability of self-assembled monolayers on electropolished L605 cobalt chromium alloy for stent applications. *Journal of biomedical materials research. Part B, Applied biomaterials*. 2011;98B(2):280-9.

18. Mani G, Macias CE, Feldman MD, et al. Delivery of paclitaxel from cobalt-chromium alloy surfaces without polymeric carriers. *Biomaterials*. 2010;31(20):5372-84.

19. Tegoulia V, Cooper S. Staphylococcus aureus adhesion to self-assembled monolayers: effect of surface chemistry and fibrinogen presence. *Colloids and Surfaces B: Biointerfaces*. 2002;24(3-4):217-228.

20. Zhao H, Humbeeck JVAN, Leuven KU, Sohier J, Scheerder IDE. Electrochemical Polishing of 316L Stainless Steel Slotted Tube Coronary Stents : An Investigation of Material Removal and Surface Roughness. *Progress in Biomedical Research*. 2003;8(2):7-9.

21. Gibson I, Rosen DW, Stucker B. *Additive Manufacturing Technologies*. Boston, MA: Springer US; 2010.

22. Mani G, Torres N, Oh S. Paclitaxel delivery from cobalt-chromium alloy surfaces using self-assembled monolayers. *Biointerphases*. 2011;6(2):33-42.

23. Mani G, Feldman MD, Oh S, Agrawal CM. Surface modification of cobalt–chromium–tungsten–nickel alloy using octadecyltrichlorosilanes. *Applied Surface Science*. 2009;255(11):5961-5970.

24. Kokubo T, Kim H-M, Kawashita M. Novel bioactive materials with different mechanical properties. *Biomaterials*. 2003;24(13):2161-2175.

25. Mani G, Johnson DM, Marton D, et al. Drug delivery from gold and titanium surfaces using self-assembled monolayers. *Biomaterials*. 2008;29(34):4561-73.

26. Aronsson BO, Lausmaa J, Kasemo B. Glow discharge plasma treatment for surface cleaning and modification of metallic biomaterials. *Journal of biomedical materials research*. 1997;35(1):49-73.

27. Paz Y. Self-assembled monolayers and titanium dioxide: From surface patterning to potential applications. *Beilstein journal of nanotechnology*. 2011;2:845-61.